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IMPERIAL BUREAU OF MYCOLOGY
10 AUG 1923

REPRINTED FROM
THE ANNALS OF APPLIED BIOLOGY
VOL. X, NO. 2, JULY 1923

CAMBRIDGE
AT THE UNIVERSITY PRESS

[From THE ANNALS OF APPLIED BIOLOGY, Vol, X, No. 2, July, 1923.]

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FUNGI ASSOCIATED WITH "DIE BACK" IN STONE FRUIT TREES. I.[1]

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(With Plates XI–XIV.)

INTRODUCTION.

The investigation as to the various causes of "die back" to which all stone fruit trees are so prone, was started with the study of a serious wilt and "die back" of Prince of Wales plum. This disease was first brought to the notice of the writer in 1919, by Mr Lobjoit, Controller of Horticulture. He described the disease as causing very serious damage, in Middlesex, to plum trees of the Prince of Wales variety, so much so, that there was considerable risk of this good market variety going out of cultivation. The investigation was then extended to all forms of "die back" in stone fruits, to see if "die back," in general, could be attributed to biological strains of one and the same fungus. Results, however, have shown that this is probably not the case. Four distinct fungi, easily distinguishable from one another, have been isolated from diseased tissues in "die backs" occurring on various kinds of stone fruits such as peach, apricot, nectarine, pear, different varieties of plums and also plum and apple stocks.

Three fungi only will be dealt with in this paper, namely *Cytospora* and *Eutypella*, on which a considerable amount of work has been done already by other investigators, and a new species of *Diaporthe*, which, so far, has not been recorded as being the cause of, or being associated with, "die back" in stone fruit trees in this country.

The causes of the so-called "die back" of fruit trees are still little understood, in spite of a considerable amount of literature which has accumulated on the subject. There appears to be a good deal of confusion owing to different investigators ascribing "die back" on different hosts to different organisms, and also to the fact that most, if not all of the

[1] A grant in aid of publication has been received for this communication.

fungi described, are alleged to have two distinct stages in their life-history, namely a pycnidial stage, in the form of *Phomopsis*, *Fusicoccum*, *Phoma* or *Cytospora*, and a perithecial or valsoid stage.

Massee in 1902 (35) investigated "die back" in young nursery fruit trees, more especially plum, apple, and pear, and attributed the disease to the fungus *Eutypella prunastri*. He also claimed to have brought about infection by applying spores of the conidial stage to lenticels in damp weather. There is, however, considerable doubt as to whether *E. prunastri* occurs on cultivated plum in this country, and if it does, it is far from common. For the last two years, during which a considerable number of specimens of "die backs" on various hosts have been examined, no *Eutypella* has been observed on cultivated plum, peach, apricot or apple, in spite of careful search.

True *E. prunastri*, however, does occur on wild sloe, wild plum, wild cherry, crataegus, etc. (46). *Eutypella*, as described by Massee, has one-celled ascospores, whereas all the perithecia found during the present investigation, with one single exception, contained bicellular ascospores. Since Massee's publication, it seems to have been generally accepted that "die back" is caused either by *E. prunastri* or by *Cytospora leucostoma* or *C. rubescens*. Both Cooke (5) and Saccardo give *C. rubescens* as the conidial stage of *Eutypella*.

Epidemics of "die back" attributed to *E. prunastri* have been reported from time to time. In 1902, in the *Woburn Report* (60), an interesting description is given of a serious outbreak of "die back" in a plantation of young plum trees on the estate, in which 41 per cent. of the trees, from four to nine years old, were killed in the course of three to four years. Different investigators attributed the disease to different causes, including *Eutypella*. Some of the symptoms described agree very closely with those observed by the present writer in cases of "die back" due to *Diaporthe*. This outbreak seems to have been successfully stopped by cutting away all diseased parts, and by removing all dead or dying trees. In 1910 (51) another serious outbreak of *E. prunastri* was reported as occurring in Kent, Worcestershire and Herefordshire, but no description of the fungus was given.

Besides *Cytospora* and *Eutypella*, other members of the genera *Phomopsis*, *Fusicoccum*, *Phoma*, etc. with or without a known ascigerous stage, have been recorded as causing disease on widely different host plants, some—more especially in the case of hard-wooded plants—associated with "die back," others as causing rots, etc. Further extensive investigation, together with cross inoculations, alone will show

whether the organisms occurring on closely allied hosts, the different stages of which have been described by different investigators under various names, may not be strains of the same fungus. It is not possible to give a complete list, but references to the more important and better known diseases caused by fungi belonging to, or closely allied to, the above genera, are given in the bibliography.

(1) CYTOSPORA.

After years of controversy (1, 14, 16, 17, 34, 55, 56), between pathologists in Germany, there appears to be no doubt that Aderhold (1) was able to prove by inoculation experiments that a *Cytospora*, with a valsoid stage, having gained access as a saprophyte on dead twigs, etc. can spread through the living tissues as a parasite and cause "die back" of cherry in Germany. Rolfs (48) in America also made successful cross inoculations with both pycnospores and ascospores of *Cytospora* on peach, plum, apricot, red cherry, and wild cherry.

On the other hand, Wormald (62), working on cherry "die back" in this country, failed to bring about the disease by inoculation, although he always found *C. leucostoma* associated with "die back" on cherry. He also failed to find mature perithecia.

It is difficult to say whether this strain of *C. leucostoma* is the same as the *Cytospora* with the valsoid stage occurring in Germany and America, more especially as perithecia of *C. leucostoma* have not been observed so far on stone fruits in this country.

Belgrave (4) has described *Cytospora* as occurring on Victoria, Prince of Wales and Pond's Seedling plums, but he did not make inoculations with pure cultures, and was not able to find mature ascospores. He found that "die back" was followed by the formation of pycnidia with red gelatinous tendrils of the genus *Cytospora*, "the members of which are recognised as conidial stages of various species of *Valsa*."

In the opinion of the writer, it is doubtful, judging from first-hand experience, and the failure of the above workers to bring about infection by inoculation, whether, with the exception of cherry and peach, the strain of *Cytospora* found in this country associated with "die back" in stone fruits, is really the primary cause of the disease. During the present investigation, the perithecial stage of this fungus has never been found, and in no instance have cultures of ascospores from valsoid perithecia on cultivated fruit trees, given rise to the conidial stage of *Cytospora*. Valsoid perithecia do occur in considerable numbers on branches suffering from "die back" on different hosts; but these perithecia, which have

always shown two-celled ascospores, belong to *Diaporthe*, a fungus entirely distinct from *Cytospora* or *Eutypella*, both of which have unicellular acospores.

A detailed description of *C. leucostoma* is to be found in Wormald's paper(62) on "The *Cytospora* disease of the Cherry," and this description agrees in every detail, as far as the morphology of the pycnidial stage of the fungus is concerned, with the *Cytospora* observed on peach and different varieties of plum during the present investigation.

In the case of plum trees, *Cytospora* is very frequently present on the dead parts of trees suffering from "die back," but it appears to be secondary, and the cause of the mischief can generally be traced to a region lower down, where the tissues are still alive, but more or less moribund. The bark in this region may or may not show definite signs of disease, but if portions are kept under moist conditions in a petri dish, the *Phomopsis* stage of a *Diaporthe* will develop in the course of a few days. A very striking instance of this occurred at Cookham Dean, in a young plantation of standard trees of the Prince of Wales variety of plum, a considerable number of which had been killed. *Cytospora* was found on portions of the dead branches sent in for examination. The nursery was then visited and the dead and dying trees carefully examined. The trees had been grafted at soil level, and the scion taken up to form the stem of the young standard trees, and examination at the base of the stem in the region of the graft showed typical *Diaporthe* lenticel-like pustules on nearly all the trees. Chips of bark from near the graft from a number of the trees on being kept a few days under moist conditions, developed the typical *Phomopsis* stage, and in a few instances the *valsoid* stage of *Diaporthe*.

The trees had evidently died from the graft upwards, as the stocks were alive and apparently healthy.

So far, inoculations on Prince of Wales plum with pure cultures of *Cytospora* isolated from the same variety of host, made by inserting spores under the bark of one year old shoots, have given negative results. The wounds remained healthy, showed no signs of infection and healed up quite as well as the controls. Two sets of inoculations were made, one at the end of March, 1920, and the other at the end of May in the same year. The trees have now been under observation for rather more than two years.

However, so little is known, as yet, as to the conditions conducive to infection by *Cytospora* and other members of this difficult group of Valsaceae, that these negative results must not be taken as conclusive.

There are grounds for believing that *Cytospora* may be weakly parasitic and infect tissues which are not quite normal owing to other causes, such as bad cultural conditions, insufficient moisture, excessive or indifferent pruning, bad drainage, etc. or it may gain access into the host as a saprophyte living for a time on dead twigs, spurs, etc. and from thence spread to the living tissues. This was proved to be the case by Aderhold with cherry "die back" (1) and Hubert with *C. chrysosperma* on certain forest, shade, and ornamental trees (30).

There may be and probably are many strains of *Cytospora*, some more virulent than others, some capable of forming perithecia, others not, and so on. So far, no attempt has been made to differentiate between strains of *Cytospora*. One point, however, has been observed by Belgrave, Aderhold, and the present writer, and that is, the variability of colour in the tendrils or globular exudations of *Cytospora*, from deep red to pale yellowish pink. This variation does not seem to depend, as do the size, shape and length of the tendrils, on the amount of external moisture present.

Peaches, grown out of doors, when attacked by *Cytospora*, show definite cankers, accompanied by exudation of gum on the older stem and branches (Pl. XI, fig. 2). On the younger growth the fungus girdles the shoot, resulting in rapid wilt and "die back" of the parts affected.

(2) *DIAPORTHE PERNICIOSA.*

This fungus has proved to be far more prevalent on portions of various stone fruits suffering from "die back" than any other fungus so far met with. It has been isolated from "die back" on peach, peach stock, apricot, pear, apple stock type II (Malling), wild sloe, and the following varieties of plum, Prince of Wales, Pershore, Victoria, Coe's Golden Drop, Goliath, Greengage, various seedlings from the cross New Orleans × Victoria, seedlings of Victoria selfed, and other unnamed varieties sent in from various sources. Specimens of the disease have been received from Wisbech, Cambridgeshire, Surrey, Middlesex, Devonshire, Berkshire, Kent, Anglesea; and "die backs" have been reported from other fruit-growing areas.

The disease attacks trees of all ages, but the damage is more noticeable on young trees, when the disease generally kills the whole tree very rapidly after the first symptoms have appeared. After the examination of a considerable amount of material, the conclusions arrived at are: that the development of the fungus in the host tissues is slow; that infection occurs a considerable time—in some cases probably years—before

any definite external symptoms can be seen; but that, once wilting has occurred, the affected areas die very rapidly, and the fungus completes its life-history as a saprophyte on the dead wood.

The first symptoms may be either rapid wilting and browning of the leaves during the growing season; or premature yellowing and fall of the leaves in autumn.

The bark at first looks healthy, and frequently no external symptoms can be seen before wilting sets in. In other cases the bark may take on a reddish tinge, or slightly sunken areas may extend longitudinally down the stem or branch. The smaller branches or stem instead of being cylindrical are inclined to be angular, or flattened, and this appearance becomes more accentuated as the disease progresses. This angular appearance is due to the uneven development of secondary thickening in the parts affected. The development of secondary thickening is arrested in the diseased areas, whereas normal growth continues in the parts of the periphery unaffected by the fungus. Pl. XI, fig. 5 shows a cross-section of a small branch, cut in December, in which the previous summer's secondary thickening has developed on one side only, and has been arrested round the rest of the stem. Infection in this case must have taken place during the previous winter or early spring.

Very frequently a considerable amount of callus is formed at the edges of the healthy tissue; this is most marked when the callus is formed on both sides of the sunken area, with the result that the bark is burst longitudinally down the stem, the wood laid bare, and the callus exposed, thus increasing the risk of secondary infection by other fungi and bacteria. Longitudinal splits of considerable length are of common occurrence on the branches of Prince of Wales plum and on the stems of young standard trees when grafted at soil level.

As the disease develops, transverse elongated excrescences form on the bark, which to the naked eye are very similar to, but much more numerous than, the undeveloped lenticels on healthy bark (Pl. XII, fig. 1). These excrescences are caused by the development of stromata of mycelial tissue immediately beneath or in between the external layers of cork. The bark is finally ruptured by the rapid growth of the stroma, and the spores are liberated from pycnidia embedded in the stroma, in the form of mucilaginous threads or tendrils, white or whitey buff in colour. These tendrils vary in length and thickness (Pl. XI, fig. 6). Under very moist conditions the spores may emerge as mucilaginous globules, which spread over the surface of the bark.

The spores are given off in damp weather. In dry weather the bark

does not slit open so readily and the pycnidia may be unable to liberate the spores for an indefinite period, as long as external conditions remain the same. Under dry conditions the mucilage of the extruded tendril also dries up, and the tendrils become hard and brittle, but never appear to pulverise. The mucilage can be easily re-dissolved in water. Hence the spores of this fungus, like those of *Cytospora*, are probably not wind borne, but carried on the feet of birds, visiting insects, and by wood-boring beetles, or washed down the stem, or splashed from the soil and from tree to tree during heavy squalls of rain, cf. (28).

The perithecia mostly occur in the same stroma as the pycnidia, either singly or in groups, but in some cases the perithecia lie apart from the pycnidia and their necks emerge through bark which has not been previously ruptured by a pycnidial sorus.

The fungus kills the cortical tissues, phloem, cambium, and medullary rays, and penetrates the wood, causing considerable discolouration of the xylem. If a diseased stem is cut across before it is quite dead and dry, discoloured areas can be seen in the internal tissues, and the darkened medullary rays can be traced with the naked eye. When the tissues are very dry the discolouration is not so apparent. This discolouration is not limited to any particular area, xylem of all ages can be affected. The whole of the internal wood may be affected, or the discolouration may be restricted to wedge-shaped areas or scattered blackened spots or lines in the interior of the stem (Pl. XI, fig. 5). These areas extend longitudinally for a considerable distance. There is always more or less xylem discolouration in stems attacked by *Diaporthe*, but this discolouration must not be confused with that due to pruning scars which have not completely callussed over. Different varieties of plums vary within wide limits as to their capacity for healing over pruning scars.

In the case of *Diaporthe*, black wavy strands of cells filled with dark mycelium can be traced down the stem.

As observed by Goethe in the case of *Nectria*, and Wormald (62) in *Cytospora*, the mycelium of *Diaporthe* passes vertically through the vascular system (Pl. XIII, fig. 14) and horizontally through the medullary rays (Pl. XIII, fig. 15). In the medullary rays the mycelium passes from one cell to another mostly through the pits (Pl. XIII, fig. 15). The medullary ray cells are filled with coarse brownish mycelium, which becomes very much attenuated when passing through the pits; but although mycelium, both coarse and fine, frequently occurs in considerable quantity in the lumen of the vessels, the latter are rarely completely blocked by the fungus itself. Some of the older vessels are blocked with tyloses and

yellow deposits, probably of the nature of wound gum, but the number of vessels affected is not large enough to account for the sudden wilt. The walls of the cells are discoloured, and show a certain amount of disorganisation; in the case of the vessels, the mycelium is able to penetrate the cell wall. The wilting and death of the shoot is due to the destruction of the phloem, cambium, and cortical tissues, and the disorganisation and blocking of the medullary rays, and not to the mechanical blocking of the vessels by the fungus itself.

The Fungus.

Diaporthe is an Ascomycete belonging to the Sphaeriaceae group of the Pyrenomycetes; family Valsaceae; with two distinct stages, the *Phomopsis* of pycnidial stage, and the *Valsa* of perithecial stage.

The bark of plum at an early stage consists of several layers of cork cells, and the mycelium sometimes penetrates between these layers, and forms knots of twisted hyphae, either between or immediately below these corky layers (Pl. XIII, fig. 10). These knots develop into the stromata in which the pycnidia develop. As growth proceeds a wavy line appears in the median region of the stroma. This line, the proliferous stratum of the pycnidium (Pl. XIII, fig. 10 *h*), consists of finer, more deeply staining hyphae than the surrounding coarse mycelium of the stroma. The upper and lower layers of the stroma split apart and the split forms the irregularly shaped chamber of the pycnidium, which is lined throughout with sporophores, bearing pycnospores. The number of chambers varies according to the size and shape of the stroma, but the dividing walls do not completely enclose each chamber, so that the pycnidium of *Diaporthe* can be distinguished from that of *Cytospora* which is much more definitely multilocellate.

Two distinct forms of pycnospores or conidia are found in one and the same pycnidium, as described by Diedicke and Grove (12, 18) in the genus *Phomopsis*, and by Harter and Field (25) in *Diaporthe batatis*, namely, long filiform hyalin spores (Pl. XIII, figs. 2, 7) definitely hooked at one end (rarely straight), thickening slightly in the middle region 25–28 $\mu \times 1\,\mu$, and called by Diedicke and Grove (12, 18) "b" spores, by Nietsche (43) stylospores, and by Marchal "basides" (38); and unicellular hyalin fusiform or elongate oval spores, 7–9 $\mu \times 2$–3 μ, with two large and several smaller oil drops, the "a" spores (Pl. XIII, fig. 1).

The filiform "b" spores are given off from short, subulate (Pl. XIII, fig. 6 *a*), and the "a" spores from long, simple, cylindrical, or slightly subulate sporophores (Pl. XIII, fig. 6). The filiform spores are always

produced first in the young pycnidium, to be followed later by the development of the "a" spores, hence the first tendrils consist mostly of "b" spores. Later, the two kinds of spores are found together in the same tendril, and finally, oval spores only are given off. Microtome sections have definitely shown that "a" and "b" spores can occur in the same pycnidium. The spores are given off in enormous numbers from the sporophores, and the cavity of the chamber is completely filled with abstricted spores before dehiscence occurs. It was not possible to ascertain whether each conidiophore is capable of abstricting more than one spore, or more than one kind of spore.

The ratio of length to breadth of the oval spores varies considerably according to age (Pl. XIII, fig. 1). The mature spores are wider and shorter owing to the development of two large oil drops which push out the sides and increase the width of the spore at the expense of the length. The filiform spores contain no oil drops.

The spore-tendril emerges either through a definite short-necked ostiolum at the apex of the pycnidium (Pl. XIII, fig. 11), or it may burst through the wall of the pycnidium. In the latter case the tendril is generally coarse and short as compared with the long fine tendrils, which may attain a length of 0·5 cm., given off from the ostiolum (Pl. XI, fig. 6). The tendrils, when first extruded, are whitey buff in colour, generally paler when they consist of filiform spores only; but later, when the tendrils have dried somewhat, they become yellowish in colour, and might be mistaken for the paler forms of *Cytospora* tendrils. It is difficult to say what function, if any, the filiform spores perform. Plate cultures of filiform spores gave no growth; numerous hanging drop cultures with different media were unsuccessful except in two instances. A preparation was made with acid pea-agar, 0·1 per cent. N. HCl, as the medium, and a tendril of filiform spores taken direct from the bark of Prince of Wales plum. On this medium, the filiform spores threw out lateral processes as in Pl. XIII, fig. 7, which had all the appearance of germ tubes, but unfortunately the cover slip was broken in an attempt to focus the culture under 1/12 oil immersion. Another attempt was made with the same medium; but, although spores in this case showed signs of germination, they did not advance so far as in the first instance.

There are differences of opinion as to whether these filiform bodies are really functionless spores, or merely some form of paraphyses (Reddick(45)). In stained preparations, no definite nucleus could be found in the "b" spores: the cell contents are more or less granular, and stain deeply. The absence of any definite nucleus rather points to their

being paraphyses—more especially as disintegrating "b" spores can be frequently seen in the older pycnidia.

The oval "a" spores, on the other hand, germinate quite freely on a considerable number of different media, giving rise to the typical *Phomopsis* stage with "a" and "b" spores.

The strain isolated from apricot has only been found in the *Phomopsis* stage, and differs somewhat as to its morphological characters from the strains on plum and peach. The "a" spores are rather more pointed and fusoid, 7·5–9 $\mu \times$ 2·5–3 μ, and "b" spores are longer and arcuate, 40–50 $\mu \times$ 1.

Perithecial Stage.

When the pycnidia have opened out and are quite empty, the perithecia begin to form in the deeper-seated layers of the stroma or in the cortical tissues, either below, or by the side of the pycnidia (Pl. XIII, fig. 12), and the valsoid black necks of the perithecia mostly emerge from the same lenticel-like slits from which the pycnospores have been liberated. The base of the pycnidium is pushed up by the developing perithecia, and in its turn is burst through by the growth of the necks of the perithecia. The walls of the perithecium consist of several closely packed layers of dark thick walled mycelium, enclosing several thin delicate hymenial layers. The perithecia contain numerous asci, 50–60 $\mu \times$ 7·9 μ (Pl. XIII, fig. 8), with eight two-celled ascospores, 10–17 $\mu \times$ 2·5–5 μ (Pl. XIII, fig. 3). The asci are furnished with two small lobes or pores at the apex of a short neck, which can be seen very readily when stained, *intra vitam*, with a saturated solution of erythrosin in glycerine. Each cell of the bicellular ascospore contains two large oil drops.

The necks of the perithecia are long, 1–3 mm., hairy and brownish in colour when young; black and glabrous when fully mature; bent in various directions and rather irregular in shape (Pl. XI, fig. 7), with a small pale brown area at the tip. They may occur singly, or, as is more generally the case, in groups or linear series from 3–24, according to the size of the stroma and the slit in the bark. The ascospores are liberated as whitey buff globules either through the ostiolum at the apex, or from any part of the neck. It requires moist conditions for the liberation of the ascospores from the neck of the perithecium. Thus it was found difficult to obtain pure cultures of ascospores from naturally dehiscing perithecia, owing to contamination by bacteria and various rapidly growing superficial fungi. Good cultures, however, can be easily obtained, by cutting away the diseased cortical tissues round a group of perithecia, and by

turning back the cut portion. The surrounding cortical tissues can then be carefully dissected away and the spherical bases of the perithecia exposed. The uninjured perithecia are then burst by slight pressure with a sterile needle and the ascospores are ejected as a mucilaginous globule, from which, with care, a fairly pure culture can be obtained. The ascospores appear to require a period of rest before germination. Ascospores obtained from old specimens which had been in the laboratory for many months germinated freely, whereas repeated attempts to germinate ascospores from freshly gathered material which had not completely dried out, failed.

Polymorphism of the organism on artificial media.

The forms of the stromata or pustules in which the pycnidia are developed vary considerably on artificial media. The more usual form of the pycnidium is more or less spherical, but aggregations of pycnidia may give rise to irregular masses, or at times the stroma may develop into a columnar or clavarieform structure (Pl. XI, fig. 4).

The same variation has been observed and fully described by the Marchals, and they point out that *Diaporthe perniciosa* shows "such a degree of polymorphism that one asks oneself if the types described under various names are not within the limits of the variability of this type."

Although not so marked, there is a certain amount of polymorphism in the fungus on the host plant, as to size and shape of stroma, number of pycnidial loculi, which vary with the size and shape of stroma; length of pycnidial and perithecial necks; variability in size and shape of pycnospores and ascospores according to age; presence or absence of "b" spores in the pycnidium, etc. It becomes more and more apparent that an adequate classification of this difficult group of fungi cannot be based on morphological characters and measurements of spores only.

PLATE CULTURES.

So far no medium has been found on which *Diaporthe* will go through both stages of its life-history satisfactorily. Platings of mass infections (*i.e.* numerous spores), and also single "a" pycnospore cultures, give rise very readily to the typical *Phomopsis* stage with both "a" and "b" spores on most of the media used; but, so far, perithecia have only developed from sowings of pycnospores in two cultures on sterile plum wood, and in one mono-pycnospore culture, isolated from a tendril with both "a" and "b" spores. Immature but normally developing perithecia were found in a sub-culture of the last-mentioned mono-pycnospore

culture on prune-juice-agar 69 days old. This culture had previously developed the typical *Phomopsis* stage with both kinds of pycnospores. Black stromatic patches were found embedded in the agar beneath these patches. Another sub-culture of the same mono-pycnospore strain grown on sterilised twig of plum developed mature perithecia with typical eight-spored asci and uniseptate spores. Inoculations with this strain, and yet another pycnospore culture from a different source, have brought about typical "die back" on the current year's growth of plum, and have developed perithecia quite freely on the host plant.

On the other hand, platings of mass infections of ascospores obtained by the method above mentioned, namely, that of bursting perithecia from the under side with a sterile needle, have given rather puzzling results. Out of 11 platings taken direct from perithecia, which showed no signs of contamination, six gave the typical *Phomopsis* stage with "a" and "b" spores, and five gave pycnidia with "a" spores only. The media used for these platings were prune-juice-oat-agar, and oat-agar, but the cultures showed quite definitely that the suppression or development of "b" spores does not depend on the medium. Of the six platings which gave "b" spores, three were on prune-juice-oat-agar and three on oat-agar, and the remaining five were also grown on both media. Single ascospore cultures, however, have, so far, only produced pycnidia very slowly and sparingly. The few pustules formed appeared typical, but did not dehisce readily, and in some instances it was found necessary to open the pustules with a sterile needle in order to examine the spores; "b" spores have never been found in the mono-ascospore cultures on any medium. The "a" spores in these cultures are rather undersized and the two oil drops very pronounced, presenting much the same appearance as the older spores from tendrils on the dryer portions of the bark on the host plant.

Mono-ascospore Cultures.

Some mono-ascospore strains produce no pycnidia, others only sparingly, and the general appearance of the cultures differs somewhat from the typical mono-pycnospore strains with "a" and "b" spores. The ascospores take nearly a week to germinate and the growth of the colony is somewhat slow. At first the surface mycelium is white, but later, on some media, such as oat-agar and oat-agar + 1 per cent. of glycerine, it becomes slightly tinged with pink. Blackened irregular patches of stromatic growth develop on the surface of the medium, forming a thin crust consisting of coarse dark mycelium with thick walls. The

perithecia mostly develop in the agar beneath these stromatic patches, and the necks protrude through the superficial crust. If there are pycnidia present, some perithecia may develop in the deeper seated portions of the pycnidial stroma.

The pycnospores in such cultures are generally shorter and wider than typical pycnospores and the oil drops very pronounced. No columnar development has occurred in mono-ascospore cultures.

At first it was thought that these differences indicated the presence of two distinct fungi in close association one with the other, but judging from the results as a whole the more probable explanation is that several biologic strains exist in this variable species.

In a recent publication Ém. and Él. Marchal(38) have described a winter storage rot of apples and pears caused by a new species of *Diaporthe* named by them *D. perniciosa*, with a pycnidial stage, *Fusicoccum malorum*, which appears to be of real importance to fruit culture in the vicinities of Gembloux, Huy, Brussels, etc. Besides causing rot of stored fruits, the fungus can attack unripe fruits of plum and peach; the fruits become completely covered with stromata, dry out and fall off. Cankers also occur on the stems and branches, more especially on apple and pear, more rarely on plum and cherry. Perithecia only appear when the fruits are completely mummified, and only "capricieusement" on the bark of affected trees. The *Diaporthe* causing "die back" of plum, etc. in this country agrees very closely as to its morphological characteristics with *D. perniciosa* with the exception of the dimensions of the asci. A specimen of "die back" on plum was forwarded to Marchal for comparison with his fungus, and he is of the opinion that the two organisms are the same, and that the slight differences observed are within the limits of the variability of this species.

As far as is known to the present writer, no species of *Diaporthe* has been recorded so far as causing "die back" of stone fruit trees in this country. Until this investigation is completed, however, it is thought advisable to adopt provisionally the name of *D. perniciosa* Marchal, *f. pruni*, for the forms on peach and plum, and *Phomopsis perniciosa f. armeniaca* for the form on apricot, which, as mentioned above, shows certain morphological differences.

CULTURES OF PYCNOSPORES OF *D. PERNICIOSA*.

Cultures from tendrils containing either "a" or "a" and "b" spores grow well on various artificial media, such as prune-juice-oat-agar (made with crushed oats); plum-shoot-decoction-oat-agar, plum-shoot-decoction-

agar, oat-agar, potato-agar, oat-agar + 1 per cent. glycerine, and also sterile shoots and blocks of plum wood; but less readily on potato or carrot. Large numbers of cultures isolated from different varieties of plum, apricot, peach, etc. kept under observation many months, have shown that the fungus will live a long time in pure culture.

Oat-agar. Mycelium white, felt-like, with greyish hairy pustules on the surface; submerged pustules show as black spots in the medium; medium not discoloured. In mono-pycnospore plate cultures mycelium in centre of colony raised and weft-like; pustular formation inclined to be zoned; pycnidia develop in large numbers with "a" and "b" spores; perithecia found only in one culture on this medium.

Plum-shoot-decoction-agar. Mycelium whitey-buff, fibrillose; pustules only slightly raised when single, but conglomerate pustules considerably raised above the surface of the medium, blackish, not so hairy as on oat-agar; medium discoloured reddish-brown; pycnidial development fair, not so good as on oat-agar; both kinds of spores. In mono-pycnospore cultures pycnidial formation zonal; no perithecia.

Plum-shoot-oat-agar. Mycelium white at first then turning buffish in colour, felt-like; pustules dark grey-green; colour of medium darkened. In mono-pycnospore colonies pycnidial development zonal; growth good; no perithecia.

Sterile wood. Growth sparse on heart wood; on younger wood mycelium whitish, spreading all over the wood; surface of wood blackened; pustules few, hairy, greyish-green, raised, some columnar; "a" and "b" spores; perithecia formed in two cultures.

Prune-juice-oat-agar. Mycelium loose at first, greyish; surface of medium frequently much creased; medium slightly discoloured; pustules raised, hairy, greyish-green in colour; good pycnidial development sometimes columnar; "a" and "b" spores; no perithecia.

Oat-agar + 1 per cent. glycerine. Mycelium at first white, turning pale greeny grey, rather loose; medium not discoloured; pustules raised but not columnar, hairy, greyish-green; good pycnidial development; no perithecia.

Potato. Growth poor and slow; pustules few, sometimes columnar; pycnidial development sparse.

Potato-agar. Mycelium loose, browny grey; pustules raised, hairy; medium slightly discoloured; good pycnidial development; "a" and "b" spores; no perithecia.

Sterile plum shoot. Mycelium loose, white at first, later tinged with buff; pustules hairy, raised, greyish-green; good pycnidial development; "a" and "b" spores; perithecia in one mono-pycnospore culture.

Carrot. Growth poor.

INOCULATION EXPERIMENTS.

Young Prince of Wales trees were inoculated in March and May of 1920 with various pure cultures of *Diaporthe*. A T-shaped incision, or a slit, was made in the bark as for budding, the bark raised and spores or mycelium inserted under the bark. The wound was then bound round with bast which had previously been boiled several times, and the whole covered with sheet gutta-percha, similarly treated. The hands of the operator were dipped in methylated spirit between each change of culture. The controls were treated in the same way.

The trees were inoculated on branches of the previous year's growth. So far, no typical wilt has resulted from these inoculations. The controls healed up quite healthily. The various cultures of *Diaporthe*, including the *Diaporthe* isolated from apricot, mostly showed more or less gumming and the wounds became sunken and unhealthy; inoculations with *Cytospora* isolated from Prince of Wales plum healed up quite as well as the controls. The trees have now been under observation for about 18 months.

On the other hand, the same cultures as those used for Prince of Wales plum on the young current year's growth of peach and nectarine in June, gave positive results in a comparatively short time.

(1) *Phomopsis* stage of *Diaporthe* isolated from bark of Prince of Wales on prune-juice-oat-agar 71 days old, produced rapid wilt on nectarine "Elruge" in 25 days (Pl. XIV, fig. 1 (2)); two inoculations, both positive.

(2) *Phomopsis* stage of *Diaporthe* isolated from bark of Coe's Golden Drop on oat-agar, 32 days old; rapid wilt on peach (var. unknown) in 25 days (Pl. XIV, fig. 1 (4)); three inoculations all positive.

(3) *Phomopsis* stage of *Diaporthe* isolated from bark of apricot on prune-juice-oat-agar, 21 days old; two inoculations with spores, on peach (var. unknown) positive in 64 days (Pl. XIV, fig. 2 (1)); and a third with mycelium as inoculum, positive after one year and 24 days.

(4) *Phomopsis* stage of *Diaporthe* isolated from bark of peach (var. unknown) on prune-juice-oat-agar, 44 days old, wilt in 17 days; two inoculations, both positive.

(5) *Phomopsis* stage of *Diaporthe* isolated from the bark of apricot on prune-juice-oat-agar, 52 days old; on young current year's growth of apricot, var. Breda; one inoculation positive after about one year.

(6) *Phomopsis* stage of *Diaporthe* isolated from the bark of Prince of Wales plum, on prune-juice-oat-agar, 73 days old; on young current year's growth of apricot, var. Kaisha; one inoculation positive after ten months.

(7) *Phomopsis* stage of mono-pycnospore culture of *Diaporthe* isolated from bark of Prince of Wales plum, on oat-agar, 18 days old (mycelium used as inoculum); on Jefferson plum; one inoculation positive after 61 days. (N.B. This mono-pycnospore culture produced perithecia on the host plant in three months.)

(8) Same culture as in (7) on young current year's growth on peach seedling (Royal George selfed); one inoculation positive; shoot dead after 11 months.

The above results show that the period which elapses between infection and the onset of the disease is very variable, and probably depends more upon the age and succulence of the parts infected, than the age of the cultures used.

Young peach shoots are more succulent than either plum or apricot. The above inoculations on the current season's growth of the various hosts were made during the months of June and July.

The organism was re-isolated from the tissues above and below the point of inoculation in at least one specimen of each of the above cases. These results prove quite definitely that all the above strains of *Diaporthe* are parasitic and cause "die back" on peach, nectarine, apricot and plum. The failure to bring about wilt on Prince of Wales plum is probably due to the inoculations having been made on older wood, and the organism has not yet permeated the tissues sufficiently to cause wilt.

Unripe apples of the varieties Lord Suffield, Peasgood Nonsuch and Bramley Seedling (Pl. XII, fig. 4) were inoculated through an incision in the skin, with mono-ascospore and mono-pycnospore cultures of *Diaporthe* isolated by the author from "die back" on Prince of Wales plum and Victoria seedling, and with a monospore culture of *D. perniciosa* forwarded by Prof. Marchal. Mycelium was used for the inocula in the case of Marchal's culture and the mono-ascospore cultures, and spores in the case of the pycnospore cultures. Decided patches of rot, identical in appearance, resulted in a few days from inoculations with plum ascospore and Marchal's cultures on Lord Suffield apples, and by plum pycnospore and Marchal's cultures on Peasgood Nonsuch. Bramley Seedling, a later apple, which was consequently still very hard and green, proved more resistant, and 14 days after inoculation only showed slight traces of rot.

Inoculations were also made on fruits of Victoria plum (Pl. XII, fig. 5) in various stages of ripeness from green fruits to those showing colour, with Marchal's culture and various other mono-ascospore and mono-pycnospore cultures. Marchal's culture proved to be the most virulent, giving rise in a few days to dark patches of rot $\frac{1}{2}$–$\frac{3}{4}$ inch in diameter, on

all the fruits inoculated. The patches of rot caused by the author's cultures appeared somewhat paler in colour. When pycnospores were used as inoculum the rot did not develop so rapidly, probably owing to the time required for the germination of the spores.

All the controls remained healthy.

The recent investigations of Wiltshire(59) as to the causes of "die back" have given interesting results. He describes the presence of bacterial masses in the diseased cortex together with fungal hyphae, the latter, in some instances developing cytospora-like fructifications, and suggests that there may be some relationship between the two organisms.

So far he has been unable to grow the bacteria on artificial media, and gives no description of the accompanying fungus. But it is quite possible that his work may throw light on the problem of the parasitism of *Cytospora*, and may show that concomitant infection with other organisms may be necessary in order to enable *Cytospora* to develop as a parasite in the host tissues.

The present writer has also observed rod-shaped bacteria in the disorganised cortical tissues attacked by *Diaporthe*; but, seeing that inoculations with *D. perniciosa* in pure culture have given definite results, more especially on peach, that they have caused considerable disturbance in one year old wood of plum, and also that they have set up rot in unripe apple and plum fruits, no concomitant organism appears to be necessary in order to bring about infection with *D. perniciosa*.

CONTROL MEASURES.

In spite of so little being known as yet as to the conditions which are conducive to infection of stone fruit trees by the fungi described in this paper, a few suggestions for control measures can be made.

Good cultural conditions and careful pruning are of prime importance. These fungi are all believed to be wound parasites and hence all cut surfaces should be treated with styptic or some antiseptic dressing. Very susceptible varieties, such as Prince of Wales plum, should be budded rather than grafted, and, if possible, well above soil level. In the case of standards the stock should be taken up to form the stem of the tree, and budded or grafted at the height required. The buds should not be taken from young, but from older trees, which have survived for a considerable number of years.

Care should be taken to examine the internal tissues of the shoots from which the buds are taken, and any shoot showing any traces of internal discolouration should be rejected.

When the bud has grown, the portion of the stock above the bud should be cut away and not left to act as a support for the growing shoot, as is so often the practice in nurseries; the cut surface should be pared smooth and treated with styptic. The same precautions hold good in the case of grafting, and the internal tissues of the stock also should be above suspicion.

Nothing is known as yet as to whether the variety of stock has any influence on the resistance or susceptibility of the scion. The stocks should be grafted young in order to avoid any great disparity in size between stock and scion, and to enable the wound to callus over completely.

The examination of a considerable number of young standard trees attacked by *D. perniciosa* has shown that in the majority of cases infection has occurred in the region of the graft, or a large pruning scar which has not healed over completely.

As regards the treatment of older trees, all diseased or dead parts should be carefully pruned back to healthy wood and the pruning scars treated as advised above. In damp weather the various fungi sporulate freely on dead or dying wood, hence all refuse and prunings should be burnt, and not left lying on the ground to infect the soil and the surrounding trees.

Implements used for cutting away diseased portions should be disinfected before use on healthy trees, more especially after a spell of wet weather. Saws, knives, the hands of the operator, etc. can be dipped in a 3 per cent. watery solution of lysol, but the lysol should on no account be used for treating pruning scars.

Specimens of mature pycnidia have been found on the host plants from January to September, and perithecia develop promiscuously throughout the year. The numbers of perithecia found are few as compared with the excessive development of pycnidia on the affected trees. This suggests that only certain strains of pycnospores are capable of forming perithecia and that the pycnidial stage is the parasitic stage. If spraying is resorted to, it should be done throughout the summer after rain; but this is hardly to be recommended on the score of expense.

In conclusion I wish to express my thanks to Mr Lobjoit, Controller of Horticulture, and Mr E. W. Lobjoit for bringing the disease to my notice and for giving me valuable information and every facility for collecting material and inspecting trees; to Mr A. D. Cotton and Mrs Alcock of the Pathological Laboratory of the Ministry of Agriculture for their help in supplying me with specimens and information as to the incidence

of "die backs" of fruit trees in various parts of the country; to Miss E. Gunnell, Horticultural Superintendent of Devon County Agricultural Committee, for supplying me with diseased specimens; to Prof. Marchal and Mr W. B. Grove for help in identifying the fungi described; to Mr Crane of this Institution; to Mr T. Richardson, County Inspector for Berks., and lastly to A. F. Emarton, laboratory assistant, for valuable help in making numerous microtome preparations and for taking the photographs for the Plates.

SUMMARY.

1. *Diaporthe perniciosa*, first isolated by Marchal from rots in stored fruits, has proved to be parasitic and one of the causes of "die back" in stone fruit trees.

2. The various strains of *D. perniciosa* isolated from "die back" on peach and different varieties of plums have proved to be parasitic, morphologically identical, and cause definite wilt and "die back" on peach, plum, apricot and nectarine. Cross inoculations of the same organisms on two year old wood of Prince of Wales plum have so far not brought about typical symptoms of "die back," but considerable disturbance, accompanied by gumming, has occurred at the points of inoculation. A strain obtained from apricot, which shows morphological differences, has also been proved to be capable of causing "die back" on peach and apricot. All the controls remained healthy.

3. The progress of *D. perniciosa* in the host tissue is slow; infection occurs a considerable time, in some cases years, before any external symptoms appear. The mycelium permeates the internal tissues more especially the medullary rays, cortical tissues, phloem cambium, and vessels.

4. *D. perniciosa* has a pycnidial or *Phomopsis* stage with "a" and "b" spores, and a perithecial or *Valsa* stage with numerous eight-spored asci. The pycnospores emerge as whitish tendrils or globules through numerous lenticel-like slits in the bark; the perithecial necks are long and protrude mostly through the same slits as the tendrils, and the ascospores are given off either through the ostiolum at the tip of the neck of the perithecium, or may burst through any part of the neck.

5. The external symptoms are sunken areas and longitudinal slits in the bark; elongated strands of numerous lenticel-like transverse slits through which tendrils and perithecial necks emerge; rapid wilt and browning of the trees in summer, or premature yellowing and fall of the leaves in the autumn.

6. The internal tissues are discoloured and secondary thickening arrested.

7. The pycnidial stage occurs on living or more or less moribund bark, the perithecial stage on the parts which are dead.

8. *D. perniciosa* shows a certain degree of polymorphism on the host plant; on artificial media this polymorphism is much more marked.

9. The *Phomopsis* stage of *D. perniciosa* grows and sporulates well on artificial media, but perithecia develop sparingly in cultures from pycnospores.

10. Cultures of mass infections of ascospores of *D. perniciosa* have given rise in some cases to the *Phomopsis* stage with "a" and "b" spores; in others no "b" spores could be found. Mono-ascospore cultures develop the pycnidial stage very sparingly, and no "b" spores have been observed in any of the cultures. Large numbers of perithecia develop in mono-ascospore cultures on various media.

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EXPLANATION OF PLATES XI—XIV

PLATE XI.

Fig. 1. (1) Healthy stem. Prince of Wales plum. $\times\frac{3}{4}$. (2) *Phomopsis* stage of *Diaporthe perniciosa* showing tendrils of spores on Prince of Wales plum. (3) *Phomopsis* stage of *D. perniciosa* on stem of standard of seedling of Victoria plum.

Fig. 2. Canker on stem of peach seedling due to *Cytospora,* probably *C. leucostoma* var. *cincta.*

Fig. 3. Branch of seedling from Victoria plum, attacked by *D. perniciosa,* showing discolouration of internal tissues in (*b*) and (*c*); (*a*) healthy branch.

Fig. 4. Slope culture of *Phomopsis* stage of *D. perniciosa* isolated from peach, on potato + 1 % glycerine; 143 days old, showing columnar development of pycnidia. $\times\frac{2}{3}$.

Fig. 5. Sections of diseased branches of Prince of Wales plum attacked by *D. perniciosa,* showing arrested secondary thickening and discolouration of internal tissues.

Fig. 6. Tendrils of *Phomopsis* stage of *D. perniciosa* showing coarse and fine tendrils. $\times 6$.

Fig. 7. Perithecia of *D. perniciosa* on bark of Pershore plum. $\times 3$.

PLATE XII.

Fig. 1. Young tree of Prince of Wales plum attacked by *D. perniciosa* in the region of the graft. Pustules showing on the bark in the diseased area d–d_1.

Fig. 2. The reverse side of the same tree showing incomplete healing over of the graft wound.

Fig. 3. (*a*) Pycnospore culture of *D. perniciosa* isolated from peach on prune-juice-oat-agar, 24 days old. (*b*) Pycnospore culture of *D. perniciosa* isolated from Prince of Wales plum on prune-juice-oat-agar, 44 days old.

Fig. 4. Inoculations of apple fruits with *D. perniciosa* from various sources. (1) Peasgood Nonsuch; control. (2) Peasgood Nonsuch inoculated with mono-pycnospore culture from bark of Prince of Wales plum. (3) Lord Suffield inoculated with mono-ascospore culture isolated from bark of Prince of Wales plum. (4) Bramley Seedling inoculated with same culture as (2). (5) Bramley Seedling control. In (2) and (4) pycnospores were used as inoculum, in (3) mycelium.

Fig. 1

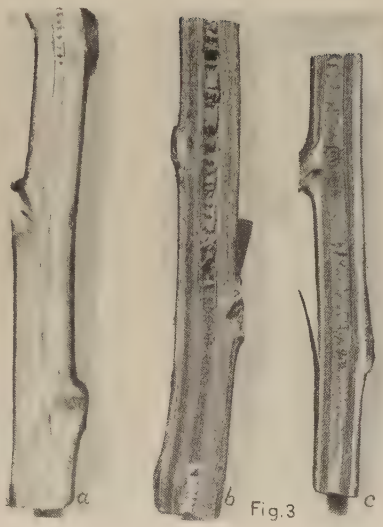


Fig. 3

Fig. 4

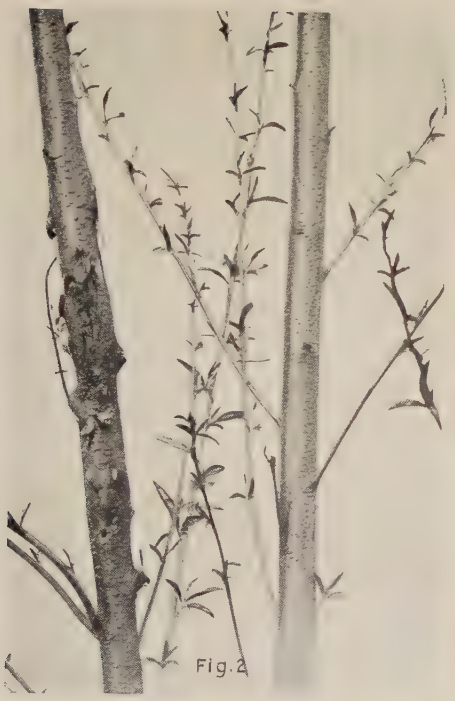


Fig. 2

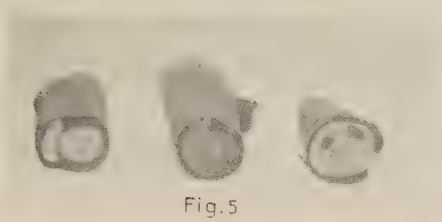
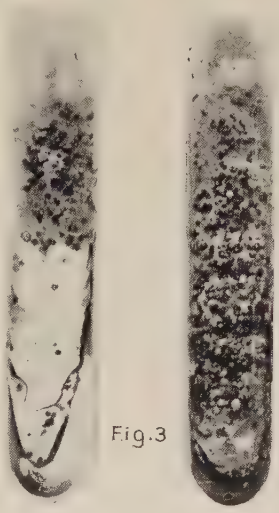


Fig. 5

Fig. 6

Fig. 7

CAYLEY.—Fungi associated with "Die Back" in Stone Fruit Trees. I. (pp. 253—275.)



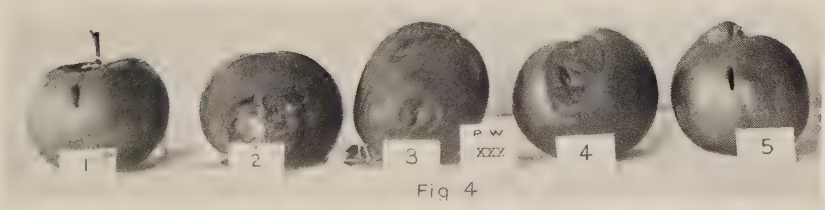
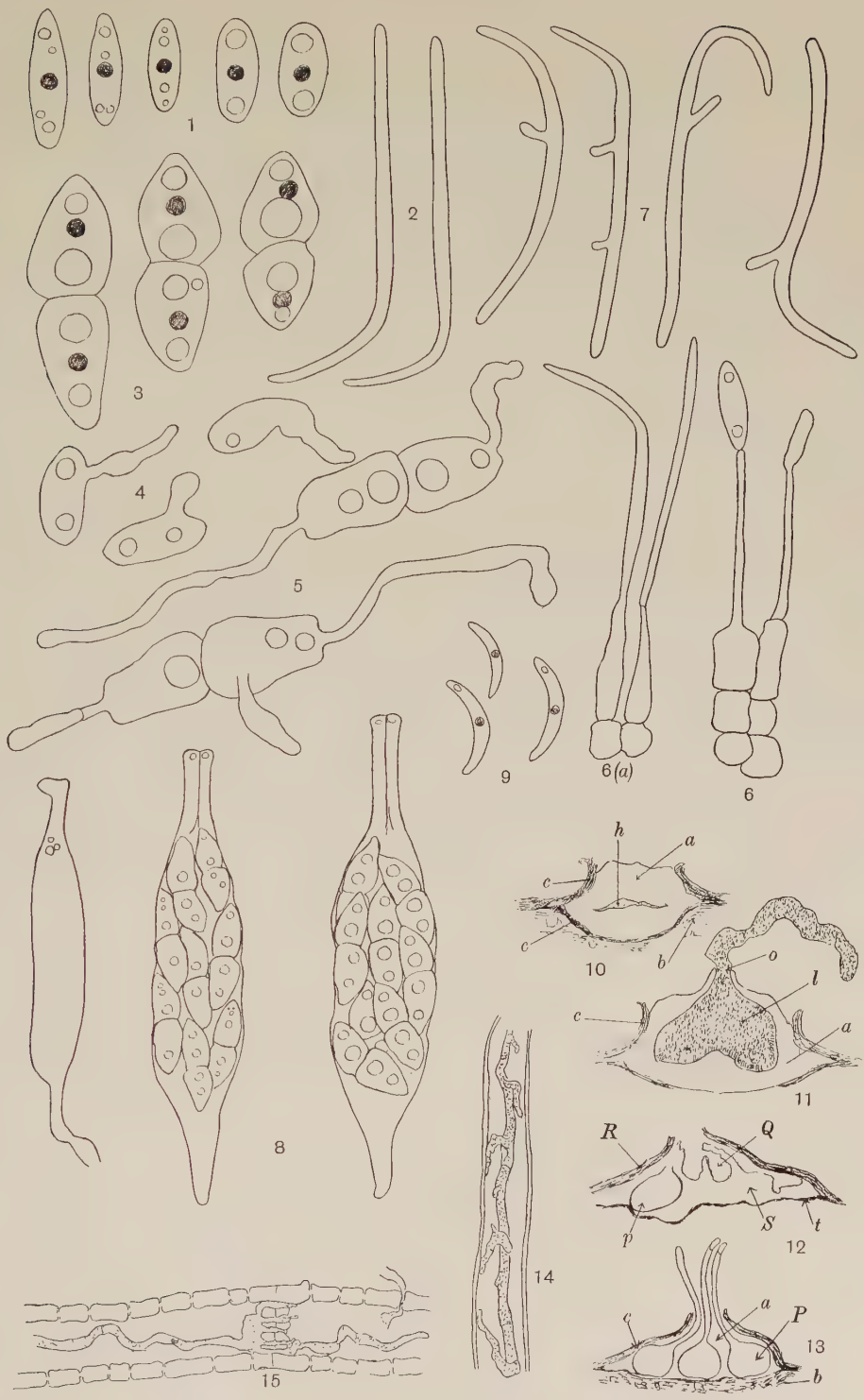


Fig 4

CAYLEY.—Fungi associated with "Die Back" in Stone Fruit Trees. I. (pp. 253—275.)



CAYLEY.—Fungi associated with "Die Back" in Stone Fruit Trees. I. (pp. 253—275.)

CAYLEY.—Fungi associated with "Die Back" in Stone Fruit Trees. I. (pp. 253—275.)

Fig. 5. Inoculation of Victoria plum fruits of varying degrees of ripeness, with *D. perniciosa* from various sources. (1) Control; ripe plum. (2) Ripe plum inoculated with multi-sporous pycnospore culture isolated from bark of Victoria plum. (3) Unripe plum inoculated with mono-pycnospore culture isolated from bark of Victoria plum. (4) Unripe plum inoculated with mono-ascospore culture isolated from bark of Prince of Wales plum. (5) Unripe plum inoculated with Marchal's mono-spore culture. In (3) pycnospores used as inoculum, in (2), (4), (5), mycelium.

PLATE XIII.

Fig. 1. "a" pycnospores of different ages. *D. perniciosa.* ×2000.
Fig. 2. "b" pycnospores. *D. perniciosa.* ×2000.
Fig. 3. Ascospores. *D. perniciosa.* ×2000.
Fig. 4. Germinating "a" pycnospores. *D. perniciosa.* ×2000.
Fig. 5. Germinating ascospores. *D. perniciosa.* ×2000.
Fig. 6. Sporophores and young "a" pycnospores. *D. perniciosa.* ×2000.
Fig. 6 *a*. Sporophores and young "b" pycnospores. *D. perniciosa.* ×2000.
Fig. 7. Germinating (?) "b" pycnospores. *D. perniciosa.* ×2000.
Fig. 8. Asci of different ages. *D. perniciosa.* ×1000.
Fig. 9. Pycnospores of *Cytospora.* ×2000.
Figs. 10, 11. Development of pycnidia in the sorus, *D. perniciosa.* ×20. (*a*) mycelial tissue of the sorus; (*b*) disorganised tissues of the cortex of the host; (*c*) corky layers of the bark; (*h*) proliferous layer of the young pycnidium; (*l*) loculus of the pycnidium; (*o*) ostiolum.
Fig. 12. Stroma of *D. perniciosa.* ×20. (*p*) young perithecium; (*Q*) disorganised chamber of empty pycnidium; (*R*) cork; (*S*) mycelial tissue of stroma; (*t*) black delimiting line of the stroma.
Fig. 13. Group of perithecia of *D. perniciosa*: (same figuring as in Figs. 10, 11).
Fig. 14. Mycelium of *D. perniciosa* passing vertically up a fibre.
Fig. 15. Mycelium of *D. perniciosa* passing horizontally through the pits of a medullary ray cell.

PLATE XIV.

Fig. 1. Inoculation on peach and nectarine. (1) Control (attacked by aphis). (2) Nectarine "Elruge" inoculated under the bark with pycnospores of *D. perniciosa* isolated from Prince of Wales plum; 26 days after inoculation. (3) Same as (2). (4) Peach (var. unknown) inoculated with pycnospores of *D. perniciosa* isolated from Coe's Golden Drop plum.
Fig. 2. (1) Peach (var. unknown) inoculated with pycnospores of *D. perniciosa*, isolated from apricot; 64 days after inoculation. (2) Control. × = point of inoculation.

(*Received November 7th*, 1922.)